A method to estimate the rate constants of DNA annealing reaction from the experimental data:

What we need:

* Time evolution data of hybridized DNA,
* Initial concentration of the primer
* Initial concentration of the single strands
* It is preferred to have equal concentration of single strands and primer.

Curve fitting

From the information of initial concentration of the single strands, it is possible to determine the conversion of the reaction and it is defined as



Based on the number of measurement, a set of *x*(*t*) can be determined. Now, at each time t, the following quantity should be calculated.



*xe* is the equilibrium conversion which can be obtained once the reaction reaches steady state. Plot *y*(*t*) vs *t*. This plot should yield a straight line and the slope of the straight line is given as



Where *k1* is the forward rate constant of the annealing reaction and *CS0* is the initial single strands concentration. Once *k1* is estimated then using the equilibrium constant, it is possible to determine the reverse rate constant *k2*.

Next Step:

Calculate the melting temperature of the given oligonucleotides using Nearest Neighbor(NN) method and verify whether the theoretically obtained melting characteristics match with the experimental values. The experimental melting characteristics suggest that it follows two states or all or none model.(only one peak)

In protocol I and II, from the time of incubation we need to monitor the duplex concentration. Due to the instruments limitation as mentioned by Sudha, there is a decrease in duplex concentration in during the start of the incubation. After some time, the concentration however increases. We will have to start our analysis from this point onwards (80 seconds from the start of the incubation). If this has to be done, what value of concentration need to be assumed at this time. Is it corresponds to the RFU value at this time or corresponds to the RFU value at the start of the incubation.

In protocol I, Compared to 74 deg C, 70 deg C produced less product for the same time of annealing. This could be due to the kinetics of the annealing reaction at two different temperatures (According to this data, 70 deg C is slow and 74 deg C is fast – But the current literature data does not support this observation). The equilibrium concentration of duplex, however, will be higher at 70 deg C and lower at 74 deg C. We will theoretically calculate the equilibrium concentration of dsDNA at these two temperatures using NN parameters and compare this with the corresponding experimentally obtained equilibrium dsDNA at these two different temperatures. This comparison will allow us to conclude which data may be corrupted.

Protocol 2 looks good. As it was mentioned before, we need to monitor the concentration of the duplex from the start of incubation till it reaches the equilibrium.

In order to differentiate the difference between protocol I and II, we need to measure the steady state dsDNA concentration or the equilibrium dsDNA concentration by allowing the reaction to reach the equilibrium. (Allow sufficiently long time so that we get saturation in RFU values. I think this has been done). Now, we can theoretically calculate the equilibrium concentration at 70 deg C or any specific temperature that we have chosen to conduct our experiment. Find the deviation between this theoretical value and dsDNA concentration that was obtained using protocol 1 and 2. This will allow us to understand whether protocol I or II is better.

The given experimental fluorescence measurements (RFU values) need to be converted in to concentration data and curve fitting needs to be done as explained above.

Karthik will do all these calculations based on the data provided by Sudha. He will get Sudha’s help to convert RFU values in to dsDNA concentration. The other concentration values such as salt concentration, primer concentration will be obtained from the ppt. If any of these data is not available, Sudha will provide them to Karthik.